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Preliminary Study of Aspartate Aminotransferase Activity in Gingival Crevicular Fluids During Orthodontic Tooth Movement

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Abstract: This study investigated the potential of Aspartate aminotransferase (AST) as a biological marker to monitor tooth movement by determining its activity in the Gingival Crevicular Fluid (GCF). Six adolescents and seven adults participated in the study. For each subject, an upper first premolar received tipping force (50-75 g) while the opposing premolar served as control. GCF was collected before force application and weekly for 4 weeks. The activity of AST was determined spectrophotometrically (30°C, 340 nm). AST activity in the GCF of test teeth in all subjects was highest at week 1 and reduced gradually in the next 3 weeks. There was a significant difference in the activity between the test and control teeth in all the subjects throughout the study ($p<0.05$). There was no significant difference in AST activity between the adult and adolescent subjects ($p>0.05$). In conclusion, AST appears to have the potential to serve as a biological marker to monitor orthodontic tooth movement.

Key words: Aspartate aminotransferase, gingival crevicular fluid, orthodontic, tooth movement, enzymes activity

INTRODUCTION

Orthodontic tooth movement involves both pathologic and physiologic responses at applied forces (Wise and King, 2008). The first response of the periodontal tissue, when a tooth is subjected to orthodontic force is acute inflammation with the release of chemical mediators and enzymes, following with remodeling of periodontal structure as a results of prolonged pressure that applied to tooth (Kyrkanides *et al*., 2000). Tooth movement is resulted from bone resorption on the compressed side and bone formation on the tension side and should proceed efficiently when the correct amount of force is applied. The formation and resorption of bone were due to increment of osteoblastic and osteoclastic cells activities. The increment of these activities can be monitored using unique biochemical marker, i.e., activities of ALP and TRAP, respectively (Intan *et al*., 2008). The duration, magnitude and type of force during orthodontic treatment have great influenced in tissue reaction. Continuous force produces concomitant bone resorption and formation at the pressure and tension areas (Bonafe-Oliveira *et al*., 2003) while intermittent force results in compression zone in periodontal ligament, short hyalinization period and lag period of resting (Krishnan and Davidovitch, 2006). A force which is too low may not be sufficient to induce tooth movement while excessive force will cause pain, attachment loss, hypermobility or halt tooth movement altogether.

The monitoring of selected biomarkers in gingival crevicular that measure the level of a biological marker which reflects the amount of force received by a tooth would be a valuable diagnostic potential tool in aiding the orthodontist to monitor correct tooth movement, i.e., improve treatment time and efficiency. When unsuitable forces are detected, i.e., either excessively or insufficient, the necessary adjustments can be made. The potential of many chemical mediators and enzymes from the Gingival Crevicular Fluid (GCF) as biological markers have been investigated (Perinetti *et al*., 2002, 2003; Asma *et al*., 2008; Rohaya *et al*., 2008). One of the basic requirements that must be fulfilled by a potential biological marker in orthodontic is that it must reflect the amount of force that is applied.

Aspartate aminotransferase (AST) is an intracellular enzyme that is released during cell death (Paolantonio *et al*., 2000). Its potential as a biological marker to monitor tooth movement has not been explored extensively although its activity had been noted

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increasing following orthodontic force (Perinetti *et al*., 2003). The initiating inflammatory event caused by the periodontal ligament microvasculature constriction resulted in focal necrosis, known as hyalinization (Murrell *et al*., 1996). Thus, periodontal inflammation following force application in orthodontics would also cause AST to be released into the GCF. Therefore, the aim of this study was to investigate the potential of AST as a biological marker to monitor tooth movement by determining its activity in the GCF after application of tipping force (50-75 g). The hypothesis was that the activity of AST in GCF would be elevated following force application.

MATERIALS AND METHODS

This study was conducted from 2006 until 2007 at Department of Orthodontics, Dental Faculty, Universiti Kebangsaan Malaysia. Six adolescents and 7 adults from a list of patients from the Department of Orthodontics, Faculty of Dentistry, Universiti Kebangsaan Malaysia have participated in the study. The mean ages of the adolescents and adults were 14.4±3.7 and 23.3±4.1 years, respectively. The inclusion criteria were need for orthodontic treatment, good general health, i.e., patients with no history of medical problems such as hypertension, diabetes or liver disease, not on medication, never been hospitalized for serious illness and good periodontal health, i.e., no alveolar bone resorption, no periodontal pockets more than 4 mm, no bleeding on probing or not more than 20% plaque score, non pregnant females, no use of anti inflammatory drugs, antibiotics or chlorhexidine mouthwash in the mouth before and during the study and written consent from patient guardian and approval from ethical committee.

In each subject, one upper first premolar either left or right was randomly chosen as the test tooth from a coin toss and its opposing lower first premolar served as the control. A transpalatal arch was constructed and cemented to the upper first molar and orthodontic brackets (0.022×0.028, Roth, Minimaster, America) were placed on the remaining upper teeth. A 0.014 inch nickel titanium (Niti preformed Ormco, CA) archwire which applied 50-75 g of force to the test tooth was placed to commence alignment and leveling while no brackets were placed in the lower arch.

GCF was collected from the mesial and distal sites of both test and control teeth. The samples were taken before placement of braces on days seven, fourteen, twenty one and twenty eight. Before collection, any plaque present was removed and sampling sites were isolated using cotton rolls and dried. A size 30 endodontic paper strip (Diadent, Group International, Korea) was inserted into the gingival sulcus at a depth of 1 mm for 30 sec. Each site was sampled 3 times at a minute interval. The samples were placed in a vial containing 100 μL Tris-HCl with pH 8.0 and stored 4°C until analyzed.

The activities in the mesial and distal sites were averaged to represent the pattern of activity of each group. GCF volume was calculated by weighing the paper points before and after collection. The weight difference between before and after sample collection was used as collected GCF volume in order to determine AST activity. Each sample was incubated for 5 min in a substrate containing 150 mmol of L-aspartate, 100 mmol of 2-oxoglutarate, 0.2 mmol nicotinamide dinucleotide (NADH), 400 mU mL^{-1} of malate dehydrogenase and 100 mmol of sodium phosphate buffer pH 7.4 in a total volume of 1.0 mL. In the presence of AST, i.e., the L-aspartate and 2-oxoglutarate exchange an amino group to produce oxaloacetate and L-glutamate. The rate of reaction was monitored by the reduction of oxaloacetate to L-malate by malate dehydrogenase with concurrent oxidation of NADH. As NADH was consumed, the change in absorbance at 340 nm was monitored at 30°C. The results were then converted to enzyme activity units (1 U = 1 μmol of NADH consumed per min at 30°C).

Data analysis was carried out using the statistical package for social sciences programme (SPSS, Version 12.0). Wilcoxon paired signed rank test was used to assess any significant difference in AST activity in GCF between the test and control samples. The confidence interval was set at 95%. The study was registered for the clinical trials with identification number ISRCTN 47483728 and was approved by the Research and Ethics Committee, Faculty of Dentistry, UKM.

RESULTS AND DISCUSSION

AST activities in the GCF of adult and adolescent subjects were highest at week 1 and gradually reduced during the following 3 weeks. The activity of the enzyme in the test teeth was higher than the control teeth throughout the four weeks of alignment (Table 1, 2).

Wilcoxon statistical analysis confirmed that there was a statistical difference between the activity in the test teeth and the control teeth in adults (Table 1) and in adolescents (Table 2) with $p<0.05$. Furthermore, it was found that there was no significant difference in the activity of the enzyme between the adult and adolescent subjects throughout the period of the study with $p>0.05$ (Table 3).

AST is an intracellular enzyme which is found in many different tissue types, with the highest levels in the heart and liver. Its increased presence extracellularly is a sign of cell necrosis (Paolantonio *et al*., 2000). When a

Table 1: AST activity (mU/sample) in GCF of adult subjects

	Test (n = 7)	Control (n = 7)	
Weeks	Median (IQR)	Median (IQR)	Significance
0	17.5(6.0)	16.6(2.0)	**
1	35.6(4.6)	17.3(2.6)	*
2	31.3(3.6)	17.3(2.6)	*
3	27.0(3.0)	17.3(2.6)	*
4	22.8(2.7)	17.2(2.6)	*

IQR: Inter quartile range; *: Statistically significant ($p<0.05$); **: Statistically not significant ($p>0.05$); Wilcoxon test showed that there was significant difference between the activities of AST in GCF of test and control teeth from 1 to week 4 in adult subjects

Table 2: AST activity (mU/sample) in GCF of adolescent subjects

	Test (n = 6)	Control (n = 6)	
Weeks	Median (IQR)	Median (IQR)	Significance
0	16.1(5.3)	15.8(4.5)	**
1	37.2(2.1)	15.9(4.5)	*
2	32.8(1.8)	15.8(4.5)	*
3	29.7(5.1)	15.9(4.5)	*
4	24.8(2.9)	15.9(4.5)	*

IQR: Inter quartile range; *: Statistically significant ($p<0.05$); **: Statistically not significant ($p>0.05$); Wilcoxon test showed that there was significant difference between the activities of AST in GCF of test and control teeth from 1 to week 4 in adolescent subjects

Table 3: AST activity (mU/sample) in GCF of adult compared with adolescent subjects

	Adult (n = 7)	Adolescent (n = 6)	
Weeks	Median (IQR)	Median (IQR)	Significance
0	17.5(6.0)	16.1(5.3)	**
1	35.6(4.6)	37.2(2.1)	**
2	31.3(3.6)	32.8(1.8)	**
3	27.0(3.0)	29.7(5.1)	**
4	22.8(2.7)	24.8(2.9)	**

IQR: Inter quartile range; *: Statistically significant ($p<0.05$); **: Statistically not significant ($p>0.05$); Wilcoxon test showed that there was no significant difference between the activities of AST in GCF of adult and adolescent subjects throughout the experiment

tooth is subjected to orthodontic force, its periodontal tissues will respond to the mechanical stress by becoming acutely inflamed due to cell necrosis, releasing various chemical mediators and enzymes including AST.

In this study, a force of 50-75 g was applied to the test teeth to induce tipping movement as the teeth start to align. In order for a tooth to tip, there must be area of compression where bone is resorbed and area of tension where bone is deposited. Bone resorption occurs in the area where the root moves toward and bone is formed in the area where the root moves away from. Focal cell necrosis is found in both areas but is more dominant in the compressed area.

This study was designed to determine the activity of AST in GCF in order to investigate its potential as a biological marker to monitor correct tooth movement during orthodontic treatment. In order to be a suitable biological marker, one of the most important and basic criteria that must be fulfilled by an enzyme is that its activity should represent the force received by a tooth at that particular time. It is a known fact that the force is highest immediately upon application and reduces gradually with time. Therefore, if AST is to serve as a biological marker, its activity should increase significantly following force application and reduces gradually thereafter.

In this study, the activity of AST in GCF of the test teeth increased significantly ($p<0.05$) and was noted at the highest level on day 7 after tipping force of 50-75 g was applied compared to the activity in the control teeth which remained constant throughout the study. Subsequently, the next 3 weeks (14, 21 and 28 days) showed that the activity of the enzyme gradually reduced although still significantly ($p<0.05$) higher than the activity in the control teeth.

Therefore, these findings showed that the increase in activity of AST in the GCF had reflected the orthodontic force level applied in the first 4 weeks. The pattern of AST activity in the first 4 weeks can also be explained by the usual practice of reactivating the appliance every 4-6 weeks as it is believed that the force becomes too low to induce anymore tooth movement after this period of time.

There had been no prior comparisons between the activity of AST in the GCF of adolescents and adults. This study found that there was no difference between the two groups although some may expect the activity in the adolescents to be higher since their periodontal cells are more active. Although the sample size were rather small for this comparison, it could possibly be explained by the fact that the force (trauma) applied to the test teeth in both groups was similar. Therefore, the degree of cell necrosis and acute inflammation were also similar thus, there was no significant difference in the AST activity.

The activities of the enzyme at the mesial and distal of test tooth were averaged to maximise the amount of collected sample. In the study done by Perinetti *et al.* (2003) the mesial and distal showed different activities due to the distally directed force. Present study concentrated on the levelling and alignment where the tipping force does not have specific direction.

Other requirements for a biological marker that can be used as biodiagnostic kit include easy collection, non invasive collection method, sample not easily contaminated, stable, easy to test and cheap processing cost. The collection of GCF was non invasive, quick and pain free. The process of enzyme testing was straight forward, not time consuming and finally the enzyme remained stable following collection and storage.

However, clinicians should note that orthodontic force is not the only factor that can increase AST

production in GCF. Generally, GCF is produced in low quantity by healthy periodontal tissues but its level increases in the presence of gingival inflammation. Thus, in this study the oral hygiene had been monitored closely before and during the treatment so as to make sure that the collected AST activity to be used to monitor tooth movement represents tooth movement induced by orthodontic force rather than by existing gingivitis.

It would have been better had the sample size in this study been larger. However, it was found that not many patients could commit themselves to returning for 5 consecutive weeks as part of the experiment. From the discussion above, it appears that AST does have the potential to be a suitable biological marker for monitoring correct orthodontic tooth movement. However, much still needs to be investigated such as the enzyme's activity during the critical event of higher force especially in bodily tooth movement is applied and the risk of anchorage loss increases. Undoubtedly, it is imperative that the correct orthodontic force is used throughout treatment. To this end, the use of a biological marker could greatly assist us in our quest to ensure that cases are treated safely, comfortably, efficiently and ultimately producing good results.

CONCLUSION

There was a significant difference in the activity of AST in the GCF between the test and control teeth following first few weeks of force application although there was no significant difference in the activity of AST in the GCF between adolescents and adults. AST appears to have the potential to serve as a biological marker to monitor correct orthodontic tooth movement.

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